Hue Expression and Shelf-Life Stability Studies of Natural Blue Food Color in Beverage Model Solution: *Clitoria ternatea* Extract, *Spirulina*, *Genipa americana*, and *Gardenia jasminoides* Commercially Produced Colorants

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Abstract

Color expression and stability of four naturally-derived food colors, butterfly pea, spirulina, huito, and gardenia extracts, was studied and compared to target color of FD&C Blue 1. Storage conditions include three different pH (2.5, 3.5, 4.5) and two temperatures (4^{o}C and 25^{o}C). Color and stability was measured using UV-Vis Spectrophotometry and Colorimetry. Butterfly pea showed superior stability over the other natural pigments, but did not show similar color to FD&C Blue 1. Its absorbance did not decrease drastically, nor did the L* and c* values. The h* value of butterfly pea was dissimilar to that of FD&C Blue 1's. On the other hand, spirulina showed very similar hue angle at pH 4.5, but it lacked in stability, by showing large decrease in absorbance and large increase in L* value. Spirulina, gardenia, and huito showed precipitation during incubation. Gardenia and huito had similar hue angle to each other, but showed dissimilar hue angle to FD&C Blue 1's.

Introduction

The use of food colorants in food products is important in increasing product appeal (7). However, in several cases, food colorants has been misused to conceal bad quality. The consumption of artificial food dye has been linked to the carcinogenic effect and hyperactivity in children (4). Synthetic additives usage, synthetic food colors being one of the component, led to today's food trend where everything natural is considered a healthier option. Some alternatives to synthetic colors include anthocyanins, betacyanins, lycopene, turmeric, and chlorophyll (7). These colors include red, orange, yellow, green, and very few blue hues. Even with relatively less field of application of blue hues, this color still is required in high amounts by some confectionery companies (6). Moreover, the stability of these natural colorants still need more studying in food applications (3). Some of the available natural blue sources include *Spirulina spp.*, *Gardenia jasminoides* fruit, *Genipa americana* fruit, and *Clitoria ternatea* flower.

Phycocyanin is the blue pigment derived from *Spirulina spp.* It is known as a protein dye, thus yielding its poor solubility in solutions, especially alcoholic beverages

(10). Flower of *Clitoria ternatea*, or known as butterfly pea, contains anthocyanins. It contains more than 9 types of anthocyanins, which provides the red-purple-blue hues on the extracted pigment. This pigment extract has been known for its stability in weakly acidic solutions and neutral solutions (12).

Fruit of *Gardenia jasminoides,* native to China, is a part of *Rubiaceae* family. Known as blue gardenia, the fruits contain three different water soluble pigments: crocins, iridoids, and flavonoids (13). This blue color was produced after geniposide turned into genipin through reaction with β-glucosidase. After that, genipin then reacted with neutral amino acids, producing blue colors. Blue gardenia pigments used in this experiment absorb at λ_{max} of 596 nm, after having been found to reacted with amino acid phenylalanine (2). Similarly, the fruit of *Genipa americana*, or known as huito, contains geniposide and geniposidic acid, which are the iridoids (11). Figure 5 below showed pigment in huito and its derivatives.

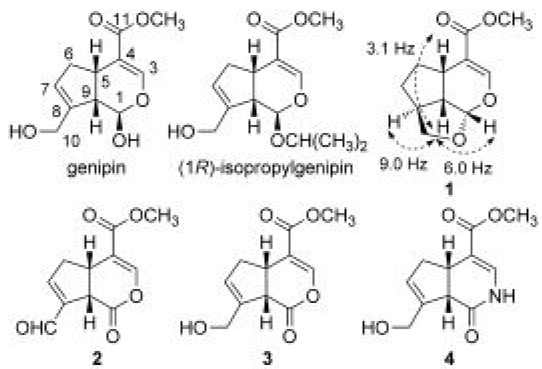

Figure 5. Structure of genipin and its derivatives (14)

This iridoids were colorless, until it reacted with primary amine with oxygen present, and formed genipin (9). Huito pigments absorbs at λ_{max} of 591 nm.

Materials and Methods

<u>Pigment extraction</u>

Pigment samples obtained were sourced from butterfly pea flowers (Amazon, Thailand), Spirulina Blue (D.D. Williamson, Louisville, KY), Acid-Stable Blue (Wild

Flavors and Specialty Ingredients, Erlanger, KY), gardenia blue extract (Yunnan Tonghai Yang, Yuxi City, China), and FD&C Blue 1 (Noveon Hilton Davis, Cincinnati, OH). Anthocyanins from butterfly pea flowers were extracted using acetone solvent extraction as described by Rodriguez-Saona and Wrolstad (5). Spirulina Blue and Acid-Stable Blue (huito-derived colorant) were obtained in concentrated liquid, while gardenia blue extract were obtained in powdered form. No extractions were required for these three sources.

Stock solutions and beverage model preparation

Pigments were diluted to 50 ml in volumetric flasks with deionized water to create stock solutions. The weight for each pigments was within 0.500±0.05 grams. Beverage model was prepared by mixing deionized water, 0.25% citric acid (Sigma Chemical Company, St. Louis, MO), 10% sucrose (Mallinckrodt, St. Louis, MO), and 0.1% sodium benzoate (Fisher Scientific, Waltham, MA). This solution was then divided into 3 pHs of 4.5, 3.5, and 2.5. The pH was adjusted using 10% KOH solution (Fisher Scientific, Waltham, MA) and 12.1 M HCl (Fisher Scientific, Waltham, MA). Amount of pigment added to the beverage model solution was standardized to the absorbance of 0.677±0.120 at pH 4.5, to avoid precipitation of some pigments. This absorbance was selected in effort to find a mid-way absorbance between 0.1 to 1.0. Each pH was stored at two different storage temperature of $4^{o}C$ and $25^{o}C$, with three replicates of sample prepared.

Data collection

Color and absorbance data were collected at day 0, 2, 4, 7, 14, 21, and 28. Color data was collected using colorimeter (Hunter Colorquest XE, Reston, VA) and absorbance was collected using UV-Visible spectrophotometer (Shimadzu UV-2450, Kyoto, Japan) from 700 nm to 380 nm. Haze readings were done weekly with identical set of samples due to late discovery of precipitate. Percent haze was measured using the same colorimeter. All samples were shaken before reading was taken to disperse any precipitate present.

Results and Discussion

Stability comparison based on pH. After incubation with three different pHs, both color and absorbance data showed little change. Change in absorbance was associated with pigment degradation. The most pigment degradation was observed at pH 3.5 (Figure 1).

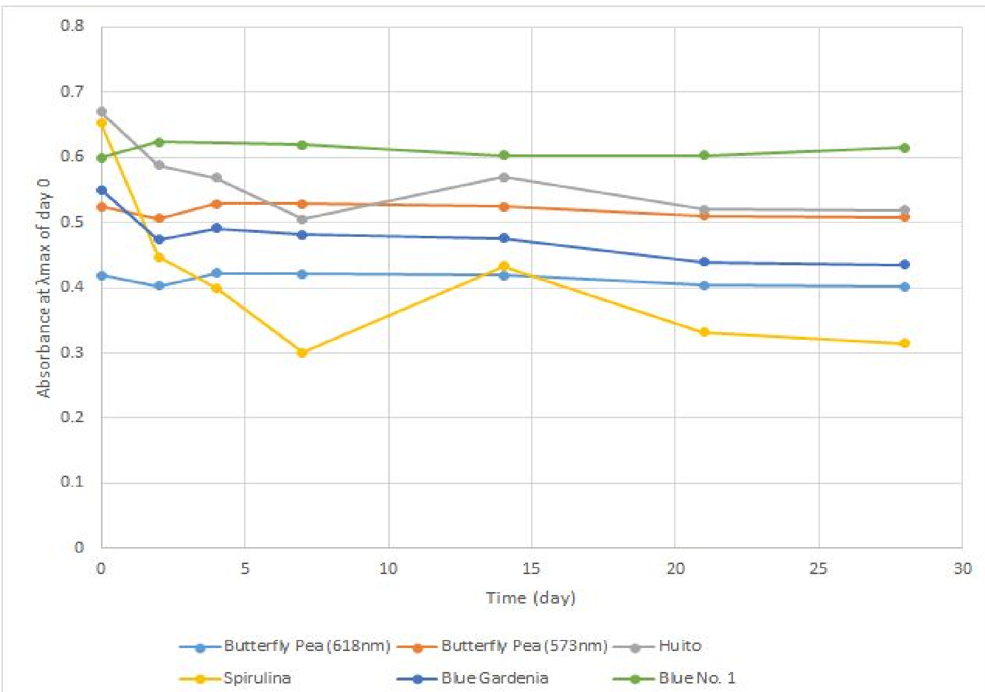

Figure 1. Change in absorbance at λ_{max} of day 0 in pH 3.5 beverage model over 28 days, incubated at 25°C

While other pigments showed very little change, spirulina showed drop in absorbance of around 0.3 and huito-derived color around 0.2. FD&C Blue 1, as a control, appeared to be the most stable, with least change in absorbance. Similarly, butterfly pea appeared to be the most stable of the natural pigments (Figure 1). In comparison between different pHs, beverage model at pH 4.5 was most stable in terms of spectral data. This can be seen further in Table 1 below.

Table 1. λ_{max} and change in absorbance at λ_{max} of day 0 across three different pH beverage model over 28 days, incubated at 25°C

Sample	pH 2.5		pH 3.5		pH 4.5	
	λ_{max} (nm)	ΔA	λ_{max} (nm)	ΔA	λ_{max} (nm)	ΔA
Butterfly Pea (618 nm)	618	0.02	618	0.02	618	0.02
Butterfly Pea (573 nm)	573	0.02	573	0.02	573	0.02
Huito-derived Color	591	0.19	591	0.15	591	0.10
Spirulina	617	0.26	617	0.34	617	0.19
Gardenia	596	0.09	596	0.12	596	0.15
Blue No.1	629	0.00	629	0.00	629	0.00

As can be seen in Table 1, butterfly pea was the most stable pigment across three pHs by having consistent difference in absorbance over 28 days. Huito-derived color and spirulina were most stable at pH 4.5, while blue gardenia at pH 2.5 (Table 1).

Looking into the color data, pH 2.5 showed most degradation over 28 days (Figure 2). Change in hue angle was associated with change in color, in this case it was blue, and inferred pigment instability or degradation.

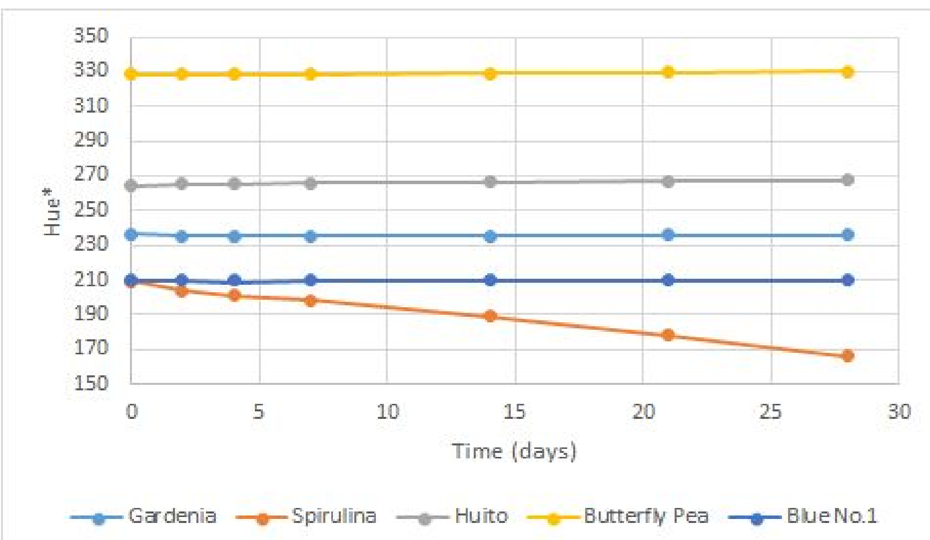

Figure 2. Change in hue angle of five different pigments at pH 2.5 after incubation in 25°C over 28 days

Spirulina showed most change in color indicated by the declining hue* number (Figure 2). However, even though other pigments showed more stability, spirulina showed the closest resemblance to control FD&C Blue 1, by having similar hue* number at point day 0 (Figure 2). Color chart was created to give visual of the color and map the positions of each pigment relative to the target color

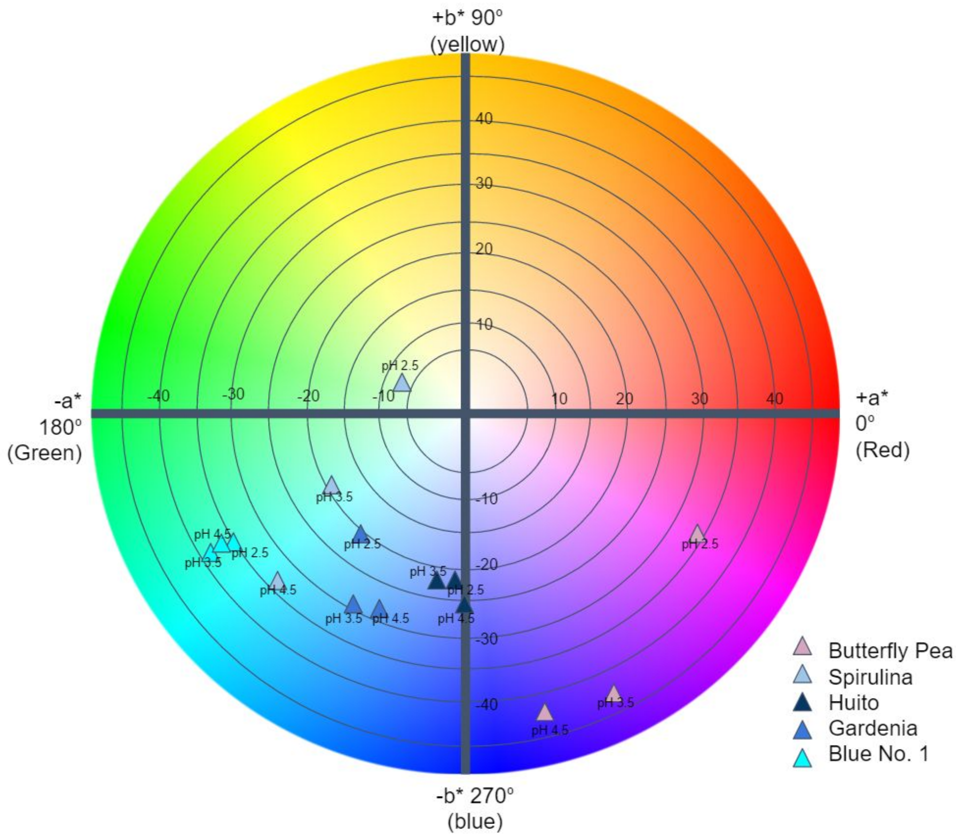

Figure 3. Color wheel showing hue angles of five food blue colorants at day 28, after incubation in 25ºC across three different pH

It can be seen from Figure 3 that spirulina at pH 4.5 was the closest to resembling FD&C Blue 1, while butterfly pea and spirulina at pH 2.5 were the furthest from target color. Further readings were collected, and displayed in Table 2 below.

Table 2. Data of L*, c*, h* numbers for five pigments over three pH at points day 0 and day 28 in format of average (standard deviation)

pH	Sample	L*		C*		H*	
		Day 0	Day 28	Day 0	Day 28	Day 0	Day 28
2.5	Butterfly Pea	63.3 (0.3)	63.0 (0.3)	36.8 (0.5)	37.5 (0.5)	328.5 (0.3)	330.3 (0.4)
	Huito-derived Color	39.6 (2.6)	43.0 (3.5)	25.9 (0.4)	22.0 (0.6)	264.4 (0.5)	267.6 (0.2)
	Gardenia	58.7 (0.9)	60.7 (2.1)	28.5 (0.7)	24.4 (1.4)	236.7 (0.4)	235.7 (0.8)
	Spirulina	79.8 (0.4)	86.8 (0.3)	25.9 (0.2)	7.8 (0.1)	209.3 (0.4)	166.4 (1.7)
	Blue No.1	80.6 (0.1)	80.0 (0.2)	40.4 (0.4)	41.6 (0.3)	209.8 (0.6)	209.6 (0.6)
3.5	Butterfly Pea	51.0 (0.8)	48.8 (0.7)	43.2 (0.8)	44.8 (0.8)	295.6 (0.3)	297.7 (0.2)
	Huito-derived Color	35.9 (0.1)	38.5 (0.2)	26.6 (0.1)	23.1 (0.1)	265.2 (0.1)	267.6 (0.4)
	Gardenia	53.2 (1.3)	54.3(2.4)	32.2 (0.4)	29.6 (0.8)	239.2 (0.2)	240.9 (0.3)
	Spirulina	66.2 (1.2)	75.0 (1.5)	41.4 (0.9)	18.8 (1.2)	227.0 (0.1)	204.0 (0.7)
	Blue No.1	80.1 (0.0)	79.3 (0.2)	41.7 (0.1)	43.2 (0.6)	211.4 (0.4)	211.4 (0.6)
4.5	Butterfly Pea	56.2 (1.4)	56.3 (1.4)	41.9 (1.1)	41.5 (1.1)	279.5 (0.8)	280.1 (0.7)
	Huito-derived Color	37.2 (0.4)	39.5 (0.4)	27.3 (0.2)	24.7 (0.2)	266.5 (0.2)	269.3 (0.2)
	Gardenia	52.4 (0.2)	54.1 (0.6)	32.9 (0.5)	29.7 (0.4)	239.0 (0.5)	242.4 (0.7)
	Spirulina	68.0 (0.2)	70.5 (0.4)	44.4 (0.2)	36.5 (0.3)	226.3 (0.2)	223.9 (0.2)
	Blue No.1	80.3 (0.8)	80.0 (0.9)	41.1 (0.7)	41.6 (1.0)	211.1 (0.7)	211.0 (0.7)

The trend of L* value were expected to increase, that was when color becomes lighter. Spirulina led the trend by having increase of 8.8 point at pH 3.5 (Table 2). This was the expected pattern. On the other side, chroma measured color intensity. The trend of chroma* numbers were expected to drop over 28 days, since color was expected to fade or lost intensity. Particularly spirulina had the largest drop of chroma* numbers. At pH 3.5, spirulina had 22.6 drop in c* value (Table 2), while other pigments had only maximum of 3.9 points drop. These two values of L* and c* worked together to represent peak instability of spirulina at pH 3.5.

The most stable pigment observed was butterfly pea because both the L* and c* values were seen to be relatively stable. The pigment showed most intensity at pH 3.5 at c* value of 44.8 (Table 2). The color data agreed with absorbance data presented above that spirulina had the least stability at pH 3.5, and butterfly pea was the most stable pigment across three pH.

Precipitation was observed at all pigment level. Pigments in spirulina and huito-derived color started showing visible precipitation at day 2, while gardenia started at day 7. Therefore, haze measurements were added into the data collection using an identical set of color samples the following week. Haze was observed the most at pH 3.5 for spirulina and huito-derived color, and pH 2.5 for gardenia. Spirulina showed very high percent haze since day 0, but did not increase at pH 2.5 (Figure 4).

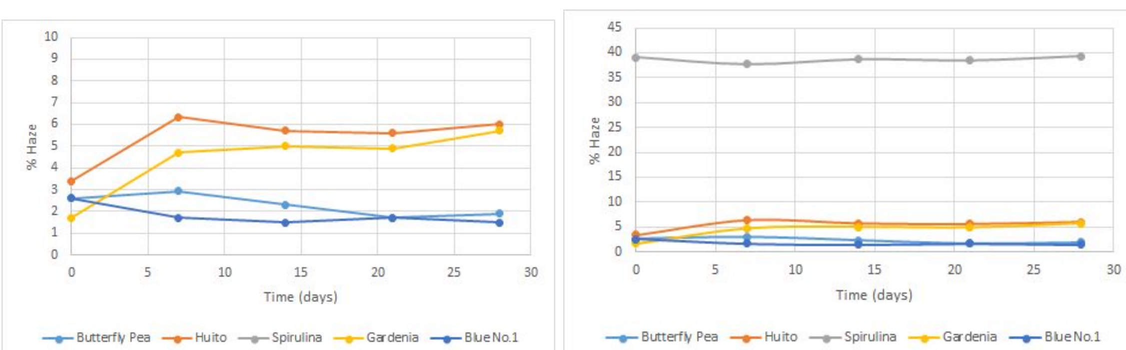

Figure 4. Percent haze over 28 days of incubation at 25°C in beverage model pH 2.5; left - without spirulina to show other pigments' progress; right - with spirulina

Figure 4 showed that huito-derived color and gardenia both increased in percent haze over time, at a similar rate likely due to their similar chemical backgrounds being iridoid compound mixtures (9). Control FD&C Blue 1 did not show any precipitation. Similar conditions were observed in butterfly pea, which had no precipitates in any pH. Spirulina showed a higher precipitation at pH 3.5, which supported previous statement about instability of spirulina at pH 3.5. The data can be seen in Table 3 below.

Table 3. Percent haze at points day 0 and day 28 on five different blue pigments across three different pH

Sample	pH 2.5		pH 3.5		pH 4.5	
	Day 0	Day 28	Day 0	Day 28	Day 0	Day 28
Butterfly Pea	2.6 (0.8)	1.9 (1.0)	2.0 (0.3)	1.4 (0.4)	2.6 (0.8)	1.8 (0.6)
Huito-derived Color	3.4 (0.3)	6.0 (0.5)	7.1 (0.8)	5.3 (0.3)	3.4 (0.3)	2.6 (0.3)
Gardenia	1.7 (0.3)	5.7 (0.3)	2.1 (0.2)	2.3 (0.4)	1.7 (0.3)	1.6 (0.3)
Spirulina	39.1 (1.2)	39.3 (2.6)	51.1 (0.2)	58.5 (2.0)	39.1 (1.2)	39.3 (0.9)
Blue No.1	2.6 (0.8)	1.5 (0.1)	2.0 (0.3)	1.2 (0.1)	2.3 (0.8)	1.7 (0.2)

As can be observed in Table 3, butterfly pea showed consistent solubility through all pH, making its solubility comparable to FD&C Blue 1. Gardenia was stable at pH 3.5 and 4.5, but at pH 2.5 it started showing precipitation towards the end of observation (day 28). It can be concluded then that butterfly pea was the most soluble, hence stable, pigment across pH 2.5, 3.5, and 4.5.

Capability to form desired blue hue. Stability and solubility tests showed one thing, but ultimately the industry is looking for the pigment that can produce the desired blue hue. Although butterfly pea demonstrated superior stability, in terms of solubility, precipitation, and absorbance, its capability to match FD&C Blue 1 was questionable. Looking back at Figure 3, butterfly pea seemed to have a purple-pink hue than blue hue. This was due to the two different peaks of absorbance it has, resulting in more coverage area and more light wavelengths absorbed. Moreover, flower extract cannot be added as natural food color according to Code of Federal Regulation (8). Spirulina showed the closest resemblance to target color of FD&C Blue 1, but with poor stability. Recommended pigment to be used would be butterfly pea considering its stability, but pH and hue expression has to be taken into consideration.

Conclusion

In conclusion, refrigerated pH 4.5 samples showed most stable results. Butterfly pea showed the most stability among the natural colorants. However, the color was not in the desired hue, but more purple. Spirulina appeared to have the closest resemblance to FD&C Blue 1, which was the target color, but with poor stability. Precipitates were also observed in pH 3.5 and 2.5 for gardenia, huito-derived color, and spirulina. In future studies, a longer period of observation should be done to calculate degradation kinetics of each pigment. Higher pH in beverage model solutions could also be made to observe better each pigment.

References

1. Downham A and Collins P. 2000. Colouring Our Foods in the Last and Next Millennium. Intl J Food Sci Technol 35: 5-22.
2. Fujikawa S, Fukui Y, Koga K, Kumada J. 1987. Brilliant Skyblue Pigment Formation from Gardenia Fruits. J Ferment Technol 65: 419-424.
3. Jespersen L, Stromdahl LD, Olsen K, Skibsted LH. 2005. Heat and Light Stability of Three Natural Blue Colorants For Use in Confectionery and Beverages. Eur Food Res Technol 220: 261-266.
4. Potera C. 2010. Diet and Nutrition: The Artificial Food Dye Blues. Environ Health Perspect 118: A428.
5. Rodriguez-Saona LE and Wrolstad RE. 2001. Current Protocols in Food Analytical Chemistry. John Wiley & Sons: F1.1.1 - F1.1.11.
6. Sigurdson GT, Giusti MM. 2014. Bathochromic and Hyperchromic Effects of Aluminum Salt Complexation by Anthocyanins From Edible Sources For Blue Color Development. J Agric Food Chem 62: 6955-6965.
7. Wrolstad RE, Culver CA. 2012. Alternatives to Those Artificial FD&C Food Colorants. Annu Rev Food Sci Technol 3: 59-77.

8. U.S. Food and Drug Administration. 2016. Code of Federal Regulation Title 21. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=101.30. Accessed 2016 November 27.
9. Newsome AG, Culver CA, Breemen RBV. 2014. Nature's Palette: The Search for Natural Blue Colorants. J Agric Food Chem 62: 6498-6511.
10. Koga K, Fujikawa S, Yuko F, inventors. 1989. Natural Blue Dye Composition and Colorant Using the Same: Prepared by reacting Taurine and Genipin. U.S. Patent US4878921A.
11. Ueda S, Iwahashi Y. 1991. Production of Anti-Tumor-Promoting Iridoid Glucosides in *Genipa americana* and its Cell Cultures. J Nat Prod 54: 1677-1680.
12. Terahara N, Oda M, Matsui T, Osajima Y, Saito N, Toki K, Honda T. 1996. Five New Anthocyanins, Ternatins A3. B4, B3, B2, and D2 from *Clitoria ternatea* Flowers. J Nat Prod 59: 139-144.
13. FMC BioPolymer. 2016. Natural Colors / Our Colors / Gardenia. http://www.fmcbiopolymer.com/Food/Ingredients/NaturalColors/OurColors/Gardenia.aspx. Accessed 2016 December 2.
14. Wang R, Zhao J, Zhang L, Peng L, Zhang X, Zheng W, Chen H. 2016. Genipin Derivatives Protect RGC-5 from Sodium Nitroprusside-Induced Nitrosative Stress. Int J mol Sci 117 (17): 1-16.